National Institute of Allergy and Infectious Diseases

BIODEFENSE WORKSHOP SUMMARY
NANOBIOLOGY STRATEGIES FOR UNDERSTANDING THE IMMUNE SYSTEM

June 21-22, 2004

Gaithersburg Marriott Washingtonian Center
Gaithersburg, Maryland

Abstract

NIAID convened a workshop on June 21-22, 2004 on nanotechnology as applied to the immune response to NIAID category A-C bioterrorism agents and the design of therapies and vaccines for those agents. Participants* presented research findings and discussed the current state of the art and recommendations in the following areas:

- Diagnostic and therapeutic applications of nanotechnology
- Basic immunology
- Materials science of nanotechnology

Topics included development of therapeutics for biodefense using nanomaterials, applications of nanotechnology to improve testing and sensitivity of immunological assays, and improved strategies for building nanostructures. The participants expressed the need for training new scientists, development and distribution of new and better reagents that could replace antibodies, development of a faster review and approval mechanism for nanobiology and related research, with review panels that are willing to consider high risk, high impact research. Furthermore, they expressed a need for NIAID sponsorship of interdisciplinary workshops and seminars to cross train biologists, immunologists, and bioengineers in the field of nanotechnology.

Introduction

Recent advances in science at the nanometer scale have made the concept of using nanotechnology for detection and sampling *in vivo* a viable proposition. Nanoobjects (e.g. nanotubes, nanofibers, nanoparticles, quantum dots) can provide a wealth of information that cannot be obtained with the same accuracy and sensitivity by any other existing methodology. Thus, the application of nanotechnology to immunological questions could greatly expand the current understanding of the immune response. Much

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* Drs. Alan Aderem, Institute for Systems Biology; James Baker, University of Michigan; Rashid Bashir, Purdue University; Jeff Carbeck, Princeton University; Harold Craighead, Cornell University; Naomi Halas, Rice University; Sang Bok Lee, University of Maryland; Chad Mirkin, Northwestern University; Timothy Mossman, University of Rochester; Lawrence Stern, University of Massachusetts; Shuichi Takayama, University of Michigan; Joyce Wong, Boston University; David Woodland, Trudeau Institute.

of the work to date in the field has been focused on aerospace and aviation, not biology or medicine. However, the areas of nanobiology and nanosystems biology are beginning to attract researchers who understand the enormous potential value of applying this technology to immunological problems, targeted drug delivery and increased accuracy and sensitivity in analyzing laboratory samples.

Diagnostics

Some of the difficulties in detecting the immune response to pathogen invasion are the amount of biological material needed to test for a response, the lack of sensitivity of the test, and the time it takes to process the material. Chad Mirkin, at Northwestern University, has developed a highly sensitive method for detecting protein analytes using nanoparticle probes that are specific to a target of interest. A complex is created from a nanoparticle probe and a magnetic microparticle probe coated with antibodies specific to the target of interest. Prostate specific antigen was the target used in the original study. Dehybridization of the oligonucleotides on the nanoparticle probe surface is performed to determine the presence of the target protein by identifying the oligonucleotides sequence released from the nanoparticle probe, in essence a biobarcode. Because the nanoparticle probe carries with it a large number of oligonucleotides per protein binding event, there is substantial amplification of the DNA, allowing detection of protein targets in the 500 zeptomolar (10^{-21}M) to picomolar (10^{-12}M) concentration range. Clinically accepted conventional assays for detecting the same targets have sensitivity limits of approximately 3 picomolar. This assay has been developed for prostate cancer, HIV, cardiac markers and Alzheimer's disease, and could be adopted for detection of pathogens used in a bioterrorist attack.

Rashid Bashir, from Purdue University, described his work in creating devices in a lab-on-a-chip format that can be used for rapid detection and characterization of cells and microorganisms for real-time analysis of nucleic acids and proteins. One such system takes advantage of the dielectrophoretic effect to sort and concentrate microorganisms within a micro-fluidic biochip allowing specific capture of particles inside the chip. Another device is used to detect the mass of bacteria and viruses as they bind to nano-mechanical silicon cantilevers. Single molecule DNA analysis using silicon-based nano-pore channel sensors can be integrated in these lab-on-a-chip devices for direct electrical characterization of these molecules expressed by a specific cell.

Therapeutics

Researchers are developing ways to use nanotechnology to deliver therapies targeted to a pathogen or virus without harm to surrounding tissue and in creating formulations that can be easily manufactured, stored, and administered.

James Baker, from the University of Michigan, presented his work in developing nanoemulsions as vehicles for vaccine and drug delivery. Nanoemulsions consist of nanometer size droplets of vegetable oil emulsified in water and stabilized with surfactants. Nanoemulsions function in a manner similar to detergents, disrupting microorganisms, but with much broader antimicrobial activity than detergents, killing not just bacteria, enveloped viruses, and fungi, but also bacterial and fungal spores and protozoa. Despite good tissue penetrance, these emulsions have better toxicity profiles than detergents because they are less efficient at disrupting tissue. This property led to their evaluation as a treatment/prophylaxis for anthrax exposure, with Phase II human toxicity trials currently underway. Investigators in Dr. Baker's lab demonstrated a protective immune response against vaccinia virus, HIV GP120/160, hepatitis-B surface antigen, and anthrax protective antigen. Experiments with anthrax protective antigen (PA) also showed that the addition of synthetic CpG oligonucleotides to the nanoemulsion protein mix could improve immune responses, so the system can potentially incorporate other adjuvants. Importantly, these results are obtained without the use of the cholera toxin, lipopolysaccharide, or bacterial proteins that are the mainstays of other mucosal adjuvants.

Another novel approach to using nanotechnology for therapeutics was presented by Naomi Halas, from Rice University. Nanoshells, developed by Dr. Halas's lab, are an example of a biocompatible nanostructure whose geometry can be modified to manipulate light in controlled ways. By adjusting the relative core and shell thickness, nanoshells can be manufactured to absorb or scatter light at a desired wavelength across visible and NIR wavelengths. Applications of these nanoshells include rapid immunoassays and optically addressable drug delivery materials. Dr. Halas also discussed her targeted cancer therapy research that is currently underway in mice. In this study, nanoshell therapy was used to treat tumors in mice. Polyethylene glycol coated nanoshells with peak optical absorption in the NIR were intravenously injected into the mice and allowed to circulate for six hours. Palpable tumors were then illuminated with a diode laser for three minutes, heating the localized nanoshells and the tumor surface. The tumors were completely cleared with no recurrence ninety days post therapy without any side effects. The immunologists on the panel speculated as to whether the therapy was stimulating the host immune system to destroy the tumor or whether the therapy itself was responsible for destruction of the tumor.

Materials Science

Construction of new nanomaterials and nanostructures are essential to the continued progress of nanotechnology into practical scientific and medical devices. Jeffrey Carbeck, from Princeton University, presented new strategies for fabrication and assembly. This work combines two existing methods, top down fabrication and bottom up assembly to pattern and orient single DNA molecules. These single DNA chains are then used as templates for the construction of hetero-structures composed of proteins and synthetic nanoparticles.

Template synthesized silica nanotubes can be potential candidates for the development of highly sensitive and selective biolabels. There are several unique properties that make nanotubes good candidates for this purpose: nanotubes can be filled with large amounts of fluorophores so that "color-coding" can take place, the walls of nanotubes are completely transparent for UV visible light to pass through, nanotubes can be easily tailored to the right size for viewing through a traditional fluorescence microscope, and nanotubes have a high number of specific binding events along their axis as compared to spherically shaped particles. Sang Bok Lee, from the University of Maryland, described his methods for loading high amounts of fluorescent dyes into the nanotubes by using hydrophobic interactions between organic and inorganic dyes and the inner surfaces of the nanotubes. He also discussed his experiments to optimize the binding affinity and specificity by lengthening the nanotubes, thus creating the potential to develop more sensitive and specific assays

Although recent advances in chip technologies, such as microarrays and protein chips, have increased our ability to rapidly identify biomarkers and make diagnoses, there are many technical challenges to overcome to increase the sensitivity and selectivity of such uses. A significant limiting factor is nonspecific binding. In selective binding, one must modify a surface in order to block nonspecific interactions and at the same time promote specific binding. Polyethylene glycol generally has been used as a tethering molecule to segregate the desired molecules from those not required for an experiment. The effects that various physical parameters have on binding are essentially unknown. Joyce Wong, from Boston University, presented her recent work on multiplexed screening of receptor-ligand binding. Her studies show that the presence of a tether significantly alters the range of receptor-ligand binding and is dependent on the dynamics of the tether. It is therefore important to be able to determine how these interactions affect the final outcome.

One of the promises of nanotechnology in biodefense or other biological applications is the potential for rapid detection and quantification of very small samples, such as those from single cells. Real time monitoring of the chemical output of the immune system is also a potential benefit. Harold Craighead, from Cornell University, discussed nanofabrication approaches to create devices for molecular detection, separation and quantification of small samples. These methods include the use of nanofluidic channels for single molecule counting and sizing, which could eventually be used to create real time immunoassays.

Shuichi Takayama, from the University of Michigan, discussed his vision for a platform for cell engineering and analysis that integrates computer controlled microfluidic cell culture and analysis with protein nanopatterned substrates that are tunable and can reversibly switch the behavior of attached cells. Such a system would be useful for the study of complex interactions among various cellular and molecular components of the immune system.

Basic Immunology

Developing treatments and vaccines in preparation for a bioterrorist attack will require a thorough understanding of how the immune system responds to different biological weapons. To help nanoengineers understand obstacles faced by the immunology community in dissecting immune mechanisms, several talks on basic immunology were presented.

During an immune response, many types of effector functions can be deployed against a pathogen. The choice of effector function is critical, as only certain mechanisms are effective against each pathogen, and unnecessary functions can cause severe host tissue damage. Cytokines secreted by T lymphocytes not only mediate some of the effector functions, but also regulate the overall type of immune response. Although considerable information has been obtained on the Th1 and Th2 T cell subtypes that secrete different sets of cytokines, it is clear that there is more T cell diversity than just these two types. Studies of T cell populations are useful but limited because T cells behave asynchronously. Furthermore, the relevant T cells in an immune response are often present at low frequencies, e.g. less than 1 in 10,000. For all these reasons, single-cell assays for multiple T cell functions are valuable, particularly if the assays can monitor sequential events in individual cells.

Tim Mossman, from the University of Rochester, discussed his modification of the standard Elispot assay into a multiparameter Fluorispot assay using fluorescence detection. Three (and potentially more) cytokines can be detected simultaneously, allowing the evaluation of complex cytokine secretion phenotypes among low frequencies of antigen-specific cells. This assay has also been combined with the Lysispot assay, also developed by Dr. Mossman's lab, that detects individual cytotoxic T cells by measuring the release of a marker protein from target cells lysed by antigen-specific T cells.

T cells play a central role in the adaptive immune response to pathogens, by directly eliminating infected cells (cytotoxicity) and by promoting antibody and inflammatory responses (helper activity). The ability of T cells to recognize and respond to foreign materials is determined by the precise reactivity of their T cell antigen receptors (TCR) with antigen peptides complexed to major histocompatibility complex (MHC) proteins present on the surfaces of other cells. Analysis of the antigen-specific T cell response is challenging, because of the low abundance of T cells responding to a particular pathogen and the relatively weak interaction of T cell receptors with their specific antigen-MHC complexes. Lawrence Stern, from the University of Massachusetts Medical School, discussed recent advances in the development of synthetic assemblies of recombinant MHC proteins in complex with defined peptide antigens. These assemblies have proven useful in the detection and analysis of antigen-specific T cells.

Macrophages represent one of the cornerstones of the innate immune system. They detect infectious organisms via a plethora of receptors; they phagocytose them, and then orchestrate an appropriate host response to them. In order to precisely define the nature of the threat, the macrophage needs to read the molecular bar code that is displayed on the specific pathogen. Recent work from a number of laboratories indicates that the family of Toll-like receptors (TLRs) plays a key role in defining the invading microorganism. Thus activation of TLR4, that detects LPS, and TLR5, that detects flagellin, would indicate the presence of a Gram negative, flagellated bacterium. This precise recognition triggers a highly regulated response to the pathogen by the host. Alan Aderem, from the Institute for Systems Biology (ISB), described how genomic and proteomic tools developed at ISB define branch points in the TLR signaling pathways. He also discussed the need for sophisticated computational methods for analyzing the increasing amounts of complex data that will be generated by nanobiology. Dr. Aderem's team has begun to address this need by creating computational methods for defining regulatory points in the TLR signaling pathways.

Respiratory viral infections induce populations of peripheral memory T cells that persist in the pleural cavity, lung parenchyma and lung airways for the life of the individual. While relatively low in number, these cells are able to mediate substantial control of secondary challenge by engaging the virus at the site of infection and when viral loads are low. Understanding the factors that regulate the recruitment and maintenance of memory T cells in different lung compartments is essential for the development of vaccines designed to promote effective cellular immunity in the respiratory tract. To better understand the regulation of T cell memory, David Woodland, from the Trudeau Institute, has been investigating the factors regulating the trafficking and maintenance of memory T cell populations in the lung. Current data suggest a dynamic process of recruitment and loss of cells with complete replacement of certain populations occurring within days. However, little is known about which subpopulations of memory T cells are specifically recruited, how they traffic into distinct compartments, or what signals are required for recruitment. Dr. Woodland outlined some technological problems associated with analysis of the very small numbers of cells needed for his research. There is a need for highly specific and sensitive methods to track low cell numbers, a need to develop methods to study trafficking *in situ*, and a need to develop methods to apply these techniques *in vivo* or in live tissue.

A recurrent theme throughout the meeting was the need for development of new types of reagents that could replace antibodies. Many nanotechnologies in biomedical applications rely on antibodies for target identification. This can pose significant limitations on sensitivity and specificity in addition to problems with cross reactivity. Creation of a standard body or repository for current approved reagents and support for the development of new types of reagents specifically for nanobiology applications are needed. In communication subsequent to the meeting, Dr. Takayama also suggested that, in addition to reagents, there is a need for support for the development of devices and materials specific to nanobiology applications and the creation of a repository of such devices and materials that could be made available to researchers.

<u>General recommendations</u>

In addition to support for research on the major topics outlined in this workshop, additional general recommendations were made on ways to advance research in this field. These include:

- Training: Emphasis on practical methods to foster collaboration between the immunology, bioengineering, nanotechnology, computer science and microbiology communities through sponsorship of interdisciplinary workshops at major meetings, cross training of postdoctoral fellows and continued forums for discussion and exchange of ideas such as this current workshop;

- Reagents: Creation of a standard body or repository for current approved reagents and support for the development of new types of reagents specifically for nanobiology applications are needed. Such reagents could include soluble labeled ligands and labeled receptor molecules;
- NIH Review: Develop a process at the NIH for faster review and approval of proposals with review panels that are willing to consider cutting edge research.

Suggested reading:

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